

## STUDIES ON SOME HEMATOLOGICAL AND SERUM BIOCHEMICAL CHANGES IN BLOOD OF SHEEP NATURALLY INFECTED WITH PIROPLASMOSIS IN SHARKIA GOVERNORATE

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### ABSTRACT

A problem of piroplasmosis was born in a flock of balady sheep, 1-3 years old from different private farms located in Zagazig, Sharkia Governorate during the period from June, 1999 to March, 2000. The effected sheep were suffering from fever, anorexia, lacrimation, corneal opacity, haemoglobinuria (urine was dark red to brown in colour), in addition to enlargement of some superficial lymph nodes nasal discharge and presence of tick infestation. After microscopical examination of blood smears, 81 out of 300 examined sheep (27%) proved to be infected with piroplasmosis. The percentages of sheep infested with babesia and theileria were 18.0% and 9.0% respectively. Haemogram of the infected animals showed microcytic hypochromic anaemia, leucopenia, neutropenia, eosinopenia, basophilia, lymphocytosis and monocytosis, while biochemical data revealed a significant decrease of total serum protein, albumin, glucose, calcium, copper and zinc. On the contrary, there was an elevation of serum total bilirubin, direct bilirubin, indirect bilirubin, iron, globulin, inorganic phosphorous, serum aspartate aminotransferase (S.AST), alanine aminotransferase (S.ALT), alkaline phosphatase (S.AP), and serum lactic dehydrogenase enzyme (S.LAD). Nearly the disturbed haematological and serum biochemical values as well as the reported clinical symptoms were returned to the normal levels after treatment.

### INTRODUCTION

Piroplasmosis is the important diseases of sheep, which is incriminated for major economic losses. Such loss result from deaths of the affected sheep, unthriftness of chronic cases and from restricted movement of animals during tick season (Medway et al., 1972). Babesiosis is a tick born disease wide spread in sheep herds within tropical and subtropical regions (McCosker, 1981). Piroplasmosis in cattle, sheep and goats belonged to three genera, Babesia, Theileria and Anaplasma. (Bucher et al., 1984). In sheep, the most common species of babesia are :

*B. ovis*, *B. motasi*, *B. eoliata* and *B. taylori*, while the respective theileria species are : *T. ovis* and *T. liri* (**Obl and Akinboada, 1984**). In Marino sheep the mortality rate due to babesiasis and theileriasis were 33% and 8.2%, respectively (**Singh et al., 1985**), while in Egypt, tropical theileriasis is widely spread disease affected chiefly the imported breeds, however, clinical infestation can occur in native breeds (**Samia et al., 1985** and **Itman, 1991**). **Selim and El-Kholany (1997)** in a survey on blood parasites in cattle and buffaloes herds in Sharkia Governorate revealed a percentage of 8.6% infected with *Babesia* and 2.1 with *Theileria*.

The principal pathogenic effect of piroplasmosis in sheep and cattle is haemolytic anemia due to intravascular haemolysis. Clinically the animal is anemic, icteric with haemoglobinemia and haemoglobinuria (**Guglicimone et al., 1996**), enlargement of some superficial lymph nodes especially prescapular and prefemoral (**Awadalla and El-kholany, 1998**). **Muller (1981)** and **Obl & Akinboada (1984)** recorded decreases in red blood cells count, packed cell volume, haemoglobin levels and total leucocyte counts, while **Srinivas et al., (1985)**, **El-Saifi et al., (1990)** and **Awadalla & El-Kholany (1998)** reported that, a marked haematological and serum biochemical changes in blood of sheep suffered from babesiosis and theileriosis were occurred in different degrees according the severity of the disease. However, research work that has been carried out in these respect is very limited and consequently little is known about the extent to which the infected sheep recover from piroplasmosis. So the present work was done to study the incidence of piroplasmosis in sheep in Sharkia Governorate and to investigate the clinical, haematological and serum biochemical changes in piroplasma infected sheep and its treatment.

## MATERIAL AND METHODS

### Animals :

The present study were carried out on 300 sheep 1-3 years old from a private farms in different localities in Sharkia Governorate, from which 81 sheep were naturally infected with piroplasmosis, in addition to 10 sheep were clinically healthy used as control group. The infected animals showing clinical signs of fever, inappetite, anaemia, with enlargement of some superficial lymph nodes especially prescapular and prefemoral, nasal discharge, lacrimation and sometimes corneal opacity, some of them showing haemoglobinurea, diarrhoea and presence of tick infestation. Both diseased and control sheep were subjected to clinical examination and daily observation in its farms. Fecal samples were collected individually from each animal and examined microscopically for detection of parasitic ova according to **Kelly (1984)**.

### Blood smears :

Thin blood smears were collected from the ear vein of all the examined animals and individu-

ally prepared. Fixed blood films were stained with Giemsa stain for identification of blood parasite according to Coles (1986). Adult ticks were collected manually from infested animals and identified according to Boudttoun et al., (1996).

#### **Blood samples:**

Two blood samples were collected from jugular vein of diseased and clinically healthy sheep before and three weeks after treatment. The first serum samples were separated after centrifugation at 3000 r.p.m for 15 minutes and collected in Vacutainer serum tubes for determination of total and direct serum bilirubin (conjugated) according to Jendrassik and Grof (1938), where indirect serum bilirubin (unconjugated free bilirubin) were determined mathematically by subtracting indirect bilirubin from total serum bilirubin. Total serum protein and albumin after the method described by Welchelbaum (1946) and Dumas (1971), respectively. Serum globulin was determined mathematically by subtracting the value of albumin from the total protein of the same sample. Serum glucose according to Trinder (1969), serum calcium according to Gindler and King (1972), serum inorganic phosphorous after Goldenberg (1966). Serum copper, zinc and iron were estimated by using atomic absorption spectrophotometer (PYE-Unicum SP-90, England) according to Khan et al., (1995). Determination of enzymatic activities of aspartate aminotransferase (S.AST) and serum alanine aminotransferase (S.ALT) according to Reitman and Frankel (1957) Serum alkaline phosphatase enzyme according to King and King (1954) and lactic dehydrogenase enzymes according to Young et al., (1975). The second blood samples was collected in heparinized vacutainer tubes for estimation of hematological RBCs, Hb and PCV% according to Schalm et al., (1975), while mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were calculated mathematically after Wintrobe et al., (1976). Total leucocytic count and its differentiation according to Benjamin (1979).

#### **\* Trials for treatment and control :**

Diazinon (Ciba-Geigy) in dilution of 1:1000 was used for eradication of ticks vectors on animals and in the surrounding areas where the animals were sprayed 3 times 5-7 days in between Babesia infected sheep was treated by Imizole (imidcarb dipropionate 12% coopers, Animal Health Ltd, England) 1ml/10kg B.W I/M together with antipyretic and terramycin L.A. (Pfizer Co. USA) 1ml / 10 kg B.W, I/M In theilaria infected sheep, the animals were treated with Arsinol 10% (El-Nasr, Pharmaceutical Chemical Co. Egypt) in a dose of 1 ml / 100 kg B.W together with antipyretic and terramycin L.A. for three successive days.

Statistical analysis of the data was performed according to Snedecor and Cochran (1980).

## RESULTS & DISCUSSION

A disease condition was appeared in a flock of sheep (1-3 years old) raised at different private farms at Sharkia Governorate during the period from June 1999 to March 2000. The present study was carried out to clarify the prevalence of babesia and theileria species among diseased sheep with piroplamosis in addition to the haematological and serum biochemical changes in blood before and three weeks after treatment. Clinical examination showed that the affected sheep were suffering from marked increase of temperature (40-41.5°C), loss of appetite and cessation of rumination. In animals with babesiosis there was sever anaemia, increased respiratory rate, laboured breathing, the urine was dark red to brown in colour. In animals with theileriosis there were serious nasal discharge, enlarged lymph nodes, lacrimation and corneal opacity in some cases. All fecal samples of diseased and healthy sheep were free from any parasitic ova. Examination of blood films of sheep revealed infection with babesia and theileria species in a percentage of 18.0% and 9.0%, respectively (Table 1). At the same time the identification of collected ticks showed the presence of *Boophilus annulatus* ticks (on sheep with babesia) and *Hyalomma anatolicum* ticks (on sheep with theileria). Haematological and serum biochemical changes before and 21 days after treatment were shown in Table (2,3). A therapeutic trials with Imizole in a dose of 3 mg/kg B.W, 1/M together with antipyretic and terramycin L.A. 1ml/10 kg B.W were effective against babesia, while Arsinol 10%, 1ml.100 kg B.W S/C together with antipyretic and terramycin L.A. were effective against theileria infected sheep. Marked improvement in haematological and serum biochemical parameters towards the normal levels were noticed after treatment of diseased sheep, in addition to the disappearance of the clinical signs (Tables 2 and 3).

Piroplasmosis is one of the most important diseases in tropical and subtropical countries. The severity of the disease increases with the long exposure to stress factors which lowers the productive performance of the animals. Examination of the fecal samples revealed that, all diseased and control sheep in this study were free from any parasitic ova due to the periodical dosing of anthelmintic drugs. Examination of blood films of diseased sheep revealed the presence of *Babesia* ovis and *Theileria annulata* in a percentage of 18.0% and 9.0%, respectively (Table. 1). *Sehim and El-Kholany (1997)* recorded nearly similar results but *Awadalla and El-Kholany (1998)* recorded lower results. This difference may be attributed to the low incidence of tick vectors. The clinical signs in sheep suffering from babesiosis were fever, loss of appetites, dry muzzle, laboured breathing, reduction of ruminal sounds, sever anaemia, lacrimation and haemoglobinuria. The clinical signs of theileriosis infected sheep were rise of temperature, serious nasal discharge, enlarged lymph nodes, lacrimation and corneal opacity in some cases. Similar signs were recorded by *Amer et al., (1987)* and *El-Sawalhy (1999)*.

Concerning the haematological changes in piroplasma infected sheep (Table. 2), it was found that there was a marked microcytic hypochromic anaemia represented by significant decrease ( $P < 0.01$ ) in the levels of total red blood cells count, haemoglobin content, packed cell volume, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Similar results were previously recorded by Radostits et al., (1995), Sahu et al., (1996) and Awadallah & El Kholany (1998). The observed anaemia was attributed to the destructive effect of the blood parasites on erythrocytes (Soulsby, 1982) or due to the toxic mediated lesion of the bone marrow and its failure to produce enough red blood cells response to compensate such losses (El-Saifi et al., 1990). The infected animals also showed leucopenia, neutropenia and eosinopenia (Table, 2). These obtained results were in accordance with those previously recorded by Pandey and Misra (1987) and Egeli (1996) in piroplasma infected sheep. The infected sheep were accompanied with lymphocytosis, monocytosis and basophilia (Table, 2). These results, were in agreement with those reported by Benjamine, (1979), and Awadalla & El-Kholany (1998). The obtained results may occur as means of body defense against infection and the formation of antibodies in response to antigens during babesia and theileria infection (Guglielmone et al., 1996).

In studying the biochemical changes in sheep infected with piroplasmosis as shown in Table (3) that total bilirubin and direct bilirubin (conjugated bilirubin) as well as indirect serum bilirubin (free serum bilirubin or unconjugated bilirubin) were increased significantly ( $p < 0.01$ ) in the infected sheep with piroplasmosis. These findings were supported by Sina and Gunay (1981) and Egeli (1996) in sheep infected with piroplasmosis as well as Cecl et al., (1997) who attributed such behavior to the destructive effect of the blood parasites to the large numbers of erythrocytes resulting in haemoglobinemia giving rise to high level of bilirubin in blood. Table (3) indicated that there was a significant decrease ( $P < 0.05$ ) in both total serum protein and serum albumin in the infected sheep when compared with healthy ones. These results were supported by the findings of Sahu et al., (1996) who attributed this decline to the destructive effect of piroplasma on the liver cells producing liver defects and impaired synthesis of total protein and albumin. Moreover, a marked hyperglobulinaemia was recorded in sheep infected with Babesia ovis due to the immune response of the animal body to the infection (El-Sawalhy, 1999). The infected sheep were accompanied also with hypoglycaemia (Table, 3). The reduction in glucose level was in agreement with those recorded by Pandey and Misra (1987) and Awadalla & El. Kholany (1998) who attributed it to the utilization of blood glucose by babesia and theileria or that diseased status giving rise to anorexia and depraved metabolic processes. On studying the effect of piroplasmosis on calcium and serum inorganic phosphorous in diseased sheep, there was a highly significant decrease ( $P > 0.01$ ) of calcium, while serum inorganic phosphorous increased

significantly ( $P < 0.05$ ) (Table 3). These findings were supported by Muley et al., (1980) and Radostits et al., 1995 who explained this findings by liver involvement and anorexia for hypocalcaemia as well as the haemolytic nature of anaemia for hyperphosphataemia. Blood serum iron level behaved a highly significant ( $P < 0.01$ ) elevation (Table. 3). This findings was supported by Pandey and Misra (1987) and El-Saifi et al., (1990) who attributed this alteration to the intravascular haemolysis. On the other hand, Table (3) showed a highly significant decrease ( $P < 0.01$ ) of both serum copper and zinc in blood of piroplasmosis infected sheep. The obtained results were in agreement with those reported by El-Saifi et al., (1990) and Cecl, et al. (1997), which may be attributed to liver and bone marrow involvement as well as copper depletion. Concerning serum enzymatic activities in blood of sheep infected with piroplasmosis, Table (3) showed a highly significant increase of serum aminotransferases (S.AST & S.ALT), serum alkaline phosphatase enzyme (S.AP) and serum lactic dehydrogenase enzyme (S.LDH) when compared with control group. These results were supported by the findings of Muller (1981) and Radostits et al. (1995). The elevated values were attributed to liver necrosis and lysis of erythrocytes during piroplasma infection.

Imizole in dose of 1ml / 100 kg B.W. I/M together with antibiotic, antipyretic and tonics were effective against babesia infected sheep. While Arsnial 10% 1 ml/100 kg B.W. I/M with terramycin L.A and antipyretic were effective against theileria infected sheep. Diazinon 1: 1000 spraying was effective for killing ticks on the infected animals.

From the present study it could be concluded that the infected sheep was suffering from microcytic hypochromic type of anaemia leucopenia, neutropenia, eosinopenia, basophilia, lymphocytosis and monocytosis. Biochemically, the elevated values of serum total bilirubin, direct and indirect bilirubin, serum globulin, serum inorganic phosphorous, serum Iron, serum enzymatic activities. Moreover, the significant decrease of total serum protein, albumin, glucose, calcium, copper and zinc, were detected in blood of sheep naturally infected with piroplasmosis. In addition to some chemotherapeutic drugs such as Imizole in a dose of 1ml/100 kg B.W together with terramycin L.A were effective against babesiosis. While Arsnial 10% in a dose of 1ml/100 kg B.W. S/C with terramycin L.A and tonics were highly effective against theileriosis as indicated by complete clinical recovery of diseased sheep and improvement of blood picture and serum biochemical parameters towards its normal levels.

Table 1 : Incidence of piroplasmosis in examined sheep.

Total No. of exam. animal	Positive cases					
	Babesia	%	Theileria	%	Total	%
300 sheep	54	18	27	9	81	27.0

Table 2 : Blood picture of sheep naturally infected with piroplasmosis and control group.

Parameter	Normal sheep (No. 10)	Piroplasmosis infected sheep (No. 81)	
		Before treatment	After treatment
R. B. C. ( $10^6/\mu\text{L}$ )	$9.25 \pm 0.75$	$6.71 \pm 0.53^{**}$	$8.95 \pm 0.23$
HB (gm%)	$11.22 \pm 0.59$	$7.22 \pm 0.24^{**}$	$10.12 \pm 0.15$
PCV (%)	$32.77 \pm 1.25$	$23.98 \pm 1.23^{**}$	$31.24 \pm 1.52$
MCV (fL)	$42.42 \pm 0.95$	$35.55 \pm 0.45$	$40.21 \pm 0.85$
MCH (Pg)	$12.55 \pm 0.88$	$10.75 \pm 0.22$	$12.22 \pm 0.18$
MCHC (%)	$32.22 \pm 0.15$	$29.15 \pm 0.75^{**}$	$31.08 \pm 0.23$
WBCs ( $10^3/\mu\text{L}$ )	$9.75 \pm 0.72$	$6.89 \pm 0.85^{**}$	$8.88 \pm 0.77$
Neutrophils ( $10^3/\mu\text{L}$ )	$29.13 \pm 0.25$	$25.23 \pm 1.25^*$	$29.07 \pm 1.02$
Eosinophils ( $10^3/\mu\text{L}$ )	$6.32 \pm 0.85$	$3.72 \pm 0.52^*$	$6.12 \pm 0.52$
Basophils ( $10^3/\mu\text{L}$ )	$0.22 \pm 0.02$	$0.92 \pm 0.01^{**}$	$0.24 \pm 0.12$
Lymphocytes ( $10^3/\mu\text{L}$ )	$63.2 \pm 5.72$	$68.00 \pm 2.55^{**}$	$61.22 \pm 1.15$
Monocytes ( $10^3/\mu\text{L}$ )	$1.12 \pm 0.05$	$2.13 \pm 0.18^*$	$1.35 \pm 0.11$

\*Significant at ( $P > 0.05$ )\*\*Highly significant at ( $P > 0.01$ )

Table 3 : Biochemical alterations in serum of clinically healthy and diseased sheep with piroplasmosis

Parameter	Normal sheep (No. 10)	Piroplasmosis infected sheep (No. 81)	
		Before treatment	After treatment
Total bilirubin (mg/dL)	0.33 ± 0.037	1.60 ± 0.15**	0.31 ± 0.05
Direct bilirubin (mg/dL)	0.08 ± 0.015	0.28 ± 0.05**	0.08 ± 0.01
Indirect bilirubin (mg/dL)	0.25 ± 0.022	1.32 ± 0.10**	0.23 ± 0.04
Total serum protein (mg/dL)	7.6 ± 0.22	6.6 ± 0.23*	7.0 ± 0.53
Albumin (gm/dL)	3.3 ± 0.15	2.0 ± 0.15*	3.0 ± 0.12
Globulin (mg/dL)	4.3 ± 0.06	4.6 ± 0.08*	4.0 ± 0.41
Glucose (mg/dL)	67.25 ± 2.72	45.75 ± 3.22**	70.0 ± 2.92
Calcium (mg/dL)	9.75 ± 0.35	6.88 ± 0.59**	10.0 ± 0.23
Inorganic phosphorus (mg/dL)	6.33 ± 0.55	7.75 ± 0.45*	6.0 ± 0.15
Copper (mg/dL)	89.75 ± 5.8	65.99 ± 6.75**	82.0 ± 5.8
Zinc (mg/dL)	98.0 ± 6.75	75.0 ± 10.57**	92.0 ± 6.0
Iron (mg/dL)	145.0 ± 8.95	235.0 ± 25.25**	142.0 ± 9.5
Serum AST (I.U/L)	62.0 ± 8.25	183.0 ± 15.22**	60.0 ± 6.8
S. ALT (I.U/L)	12.0 ± 2.15	32.0 ± 6.53**	15.0 ± 2.5
S. AP (I.U/L)	150 ± 10.75	175.0 ± 18.95**	122.0 ± 8.7
S. LDH (I.U/L)	350 ± 54.58	489.0 ± 25.87**	332.0 ± 42.0

\*Significant at (P &gt; 0.05)

\*\*Highly significant (P &gt; 0.01)



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## الملخص العربى

## دراسات على بعض التغيرات الدموية والبيوكيميائية فى دم الأغنام المصابة بالبيروبلازما فى محافظة الشرقية

## المشركون فى البحث

حمدي حسن عمران أنوار محمود عبدالعظيم

لقد ظهرت مشكلة مرض البيروبلازما فى مزارع الأغنام الخاصة والمتواجدة فى عدة قرى بمحافظة الشرقية فأجريت هذه الدراسة على ٠٠ رأس من الغنم تتراوح أعمارها بين ١- سنوات فى الفترة من يونيو سنة ١٩٩٩ حتى مارس سنة ٢٠٠٠م وبدور موضوع الدراسة عن مدى تأثير طفيل البيروبلازما على صورة الدم وكذلك التغيرات البيوكيميائية فى مصل دم الأغنام المصابة، وبالفحص الميكروسكوبى لشرايح دم الأغنام موضوع الدراسة أوضحت النتائج وجود طفيل البيروبلازما فى دم ٨١ حيوان، منها ٥٤ حالة مصابة بطفيل البابييازيس بنسبة ١٨٪ و ٢٧ حالة مصابة بطفيل الثيريازيس بنسبة ٩٪ وبالفحص الإكلينيكى للأغنام المصابة كانت الأعراض الظاهرية تشمل فقد الشهية وارتفاع درجة حرارة الجسم، إحتقان الأغشية المخاطية للعين وسرعة معدلات التنفس، ظهور البول المدمم بالإضافة إلى تضخم بعض الغدد الليمفاوية وإسهال بدرجات متفاوتة مع وجود القراد ملتصقاً فى كثير من الأغنام المصابة.

وقد أظهرت الفحوص الدموية حدوث أنيميا حادة متمثلة فى نقص العدد الكلى لكرات الدم الحمراء، والبيضا، ونسبة الهيموجلوبين وحجم خلايا الدم المضغوطة، أما المتغيرات البيوكيميائية تبين وجود زيادة ملحوظة فى المستوى الكلى للأصباغ المرارية ومستوى الصبغ المرارى الحر وكذلك الصبغ المرارى الممتد (الغير حر) كذلك لوحظ إرتفاع فى مستوى عناصر الحديد والجلوبولين والفوسفور الغير عضوى وزيادة نشاط كل من خميرة الأسبرتيت أمينوترانس فيريز (S.ALT) والألانين أمينوترانس فيريز (S.ALT) وخميرة الفوسفاتيز القاعدى (S.AP) وخميرة اللاكتك دى هيدروجينيز (S.LAD). كما وجد نقصاً ملحوظاً فى مسوى البروتين الكلى والألبومين والجلوكوز والكالسيوم والنحاس والزنك، تم عزل الأغنام المصابة وعلاج حالات البليزيا بعقار الأميزول أما حالات الإصابة بالثيليريا فقد عولجت بعقار الأرنزال مع الثيراميسين طويل المفعول وخافض الحرارة مما أدى إلى تمام الشفاء، وعودة الحيوانات لحالتها الطبيعية واتضح أيضاً إختفاء الأعراض المرضية وتحسناً فى صورة الدم والتغيرات البيوكيميائية بعد العلاج.